

Contents lists available at [SciVerse ScienceDirect](http://www.sciencedirect.com)

## Animal Behaviour

journal homepage: [www.elsevier.com/locate/anbehav](http://www.elsevier.com/locate/anbehav)

## Chemical signals of age, sex and identity in black rhinoceros

Wayne L. Linklater<sup>a,b,\*</sup>, Katharina Mayer<sup>a,c</sup>, Ronald R. Swaisgood<sup>d</sup><sup>a</sup> Centre for Biodiversity and Restoration Ecology, Victoria University of Wellington, Wellington, New Zealand<sup>b</sup> Centre for African Conservation Ecology, Nelson Mandela Metropolitan University, Port Elizabeth, South Africa<sup>c</sup> Institute of Biochemistry and Biology, University of Potsdam, Potsdam, Germany<sup>d</sup> San Diego Zoo Institute for Conservation Research, Escondido, CA, U.S.A.

## ARTICLE INFO

## Article history:

Received 11 October 2012

Initial acceptance 7 November 2012

Final acceptance 20 December 2012

Available online xxx

MS. number: 12-00786

## Keywords:

black rhinoceros

*Diceros bicornis* var. *minor*

dung

faeces

hook-lipped rhinoceros

individual discrimination

olfactory communication

signal

urine

Olfactory communication may be particularly important to black rhinoceros, *Diceros bicornis*, because they are solitary living and have comparatively poor eyesight but their populations are structured by inter- and intrasexual relationships. Understanding olfactory functions and processes might achieve better conservation management but their study in rhinoceros remains anecdotal or descriptive. Experimental approaches are required but rarely possible as rhinoceros are difficult to observe and manipulate. We measured the olfactory investigation behaviour (duration and frequency of sniffing) of black rhinoceros in four experiments designed to determine the function of chemosignals in dung and urine. A habituation–dishabituation trial demonstrated that black rhinoceros discriminated individually distinctive odours from faecal signals (experiment 1). When adults (>6 years old) were presented with dung from conspecifics of different sex and age classes (adult, and subadult from 2 to 4 years old), male dung was investigated more by both sexes, and females investigated subadult dung more (experiment 2). Both dung and urine from the same adult donors were investigated by both sexes, but male dung was investigated more than female dung and female urine more than male urine, although differences were statistically weak (experiment 3). Lastly, fresh faecal samples and those aged 1, 2, 4, 16 and 32 days were similarly investigated, indicating that they still function as olfactory signals. Together the results indicate that dung or urine signalled age, sex and identity to conspecifics and signals may persist as dung decays. Chemosignals are likely to be important to the social and spatial organization of black rhinoceros.

© 2013 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

The ability of chemical signals to persist long after the signal is deposited by an animal at a chosen location allows for information exchange among conspecifics who may not encounter one another (Alberts 1992; Wyatt 2003). Olfactory communication, therefore, is a particularly important process for solitary-living species. Physiological constraints, such as diet and endocrine state, help ensure that chemical signals remain relatively honest because the physiological by-products of these biological processes are not easily bluffed, especially in urine and dung. These waste products become rich sources of information for conspecifics, perhaps leading to the evolutionary ritualization of defecation and urination for communication (Muller-Schwarze 2006).

For communication to be efficient and effective, receivers must be able to distinguish between chemical signals. These signals govern reproductive processes in many mammalian species, facilitating mate location and selection (Bronson 1989; Wyatt 2003),

especially in solitary-living species (Fisher et al. 2003a; Swaisgood et al. 2004). Fundamental to these functions is also the ability to determine the sex and age class (i.e. adult or subadult) of the signaller from olfactory cues (Brown 1979; Mossman & Drickamer 1996), so that potential mates and competitors can be identified. For example, female mice, *Mus musculus*, covered with male urine are attacked by males and male mice covered with female urine are mounted by males, demonstrating the importance of these chemosignals for sex recognition (Dixon & Mackintosh 1971; Connor 1972). Sex-specific odours are also important for activating sexual motivation in both sexes (Wyatt 2003). Unlike other signals, scent can remain in the environment long after the signaller deposited them, making it important to determine when a chemical signal was deposited. Scent freshness might predict signaller proximity, indicating the level of threat of a competitor or the profitability of pursuing a fertile mate. The chemical constituents of olfactory signals degrade with time, providing cues that receivers can use to determine signal age (Regnier & Goodwin 1977; Roberts 1998).

One of the most fundamental and important functions of signals is to convey individual identity (Barnard & Burk 1979; Halpin 1986; Sherman et al. 1997; Tibbetts & Dale 2007). Individual recognition is a prerequisite for many social functions when there are repeated

\* Correspondence: W. L. Linklater, Centre for Biodiversity and Restoration Ecology, School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington 6140, New Zealand.

E-mail address: [wayne.linklater@vuw.ac.nz](mailto:wayne.linklater@vuw.ac.nz) (W. L. Linklater).

social interactions with the same individual such as territoriality, dominance hierarchies, kinship and mate choice. In relatively solitary and aggressive species, individual recognition could be important for identifying rivals whose competitive ability varies, providing 'cheat-proof' signals based on past history of interaction or successful territory or female defence evidenced by the spatial and temporal pattern of olfactory marks (Gosling & Roberts 2001). In 'true' individual recognition, the receiver distinguishes individually discernible cues produced by the signaller and uses them to identify the signaller in future encounters. Individual recognition lies on a continuum with other forms of social recognition that may be less precise, such as recognizing familiar versus unfamiliar, or friend versus rival. Disentangling these possibilities can be methodologically challenging, but a prerequisite to all of these functions is the ability to discriminate individually distinctive 'signatures' afforded by olfactory signals (Halpin 1986).

Olfactory communication is thought to govern many aspects of rhinoceros social behaviour. All rhinoceros species display ritualized behaviours when distributing their faeces and urine and investigating the faeces and urine of conspecifics (e.g. Goddard 1967; Schenkel & Schenkel-Hulliger 1969; Joubert & Eloff 1971; Owen-Smith 1973; Borner 1979; Laurie 1982; van Strien 1986; Kiwia 1989), similarly to other terrestrial vertebrates (see review in Alberts 1992). Other than pedal and sweat glands in some species, rhinoceros lack the specialized scent glands present in many mammalian species. The coarse diet of black rhinoceros, *Diceros bicornis*, however, produces a large amount of faecal material and they defecate approximately four to five times daily (Owen-Smith 1988), providing ample quantities for frequent olfactory signalling. Black rhinoceros defecate in communal dung heaps (Schenkel & Schenkel-Hulliger 1969) that might serve as a kind of community information exchange, as seen in other mammals (Eisenberg & Kleinman 1972). While defecating, male black rhinoceros scrape at the ground and spread their dung, but females do not ritualize defecation, suggesting that it probably functions as a male signal to competitors or potential mates. Female black rhinoceros squirt small amounts of urine frequently when they come into oestrus and male black rhinoceros spray urine over bushes and elevated objects. Both behaviours probably maximize the odour field and its detection by conspecifics, suggesting a reproductive advertisement function of urine. However, our understanding of olfactory communication in black rhinoceros, and other rhinoceros species, remains largely descriptive and anecdotal. Previous published experimental investigations of olfactory communication in black rhinoceros are limited (Goddard 1967) and, to our knowledge, there have been no such studies on any other rhinoceros species. This dearth of information is perhaps unsurprising for such a rare, difficult-to-observe, large and dangerous animal, which together limit opportunities for detailed behavioural research, but the knowledge gained may assist with conservation management for wild or captive populations (Carlstead et al. 1999; Linklater 2003, 2004; Swaisgood 2007) and contribute to our understanding of mammalian communication systems.

Research on olfactory communication has promising applications in conservation (Swaisgood 2007; Swaisgood & Schulte 2010; Campbell-Palmer & Rosell 2011). For example, studies of chemical communication in another solitary mammal, the giant panda, *Ailuropoda melanoleuca*, were instrumental in helping to turn around its conservation breeding programme (Swaisgood et al. 2000, 2004) with potential application also to the conservation of wild populations (Swaisgood et al. 2004; Nie et al. 2012). Captive breeding programmes for black rhinoceros have not sustained the population (Foose & Wiese 2006) and may also benefit from the management of communication processes. Black rhinoceros conservation programmes in the wild are improving (Linklater et al.

2011), but could also benefit from a better understanding of the species' basic ecology and behaviour. For example, translocation is an important tool for the reintroduction or restocking of populations (Linklater et al. 2012). Preliminary evidence suggests that broadcasting dung around release sites may influence postrelease settlement in newly established reserves (Goddard 1967; Linklater et al. 2006). A better understanding of the meaning of chemical signals in dung and urine will help the development of olfactory management as a conservation tool in this species.

We report an experimental approach to understanding black rhinoceros chemical communication, providing a foundation for understanding communication processes in rhinoceros and other species sharing similar life history traits. In particular, we used controlled olfactory discrimination tests with temporarily captive black rhinoceros to ask the following questions. (1) In a habituation–dishabituation paradigm (Halpin 1986), do rhinoceros show evidence of discriminating individually distinctive odours in dung? Do rhinoceros discriminate (2) the sex and (3) age of scent donors based on odours in dung? (4) Do rhinoceros also use urine as a chemical signal, as determined by differential investigation of dung versus urine? (5) Do rhinoceros discriminate dung aged for various lengths of time? As the black rhinoceros is a relatively intractable and difficult-to-study species, addressing these questions will significantly advance understanding of the species' behaviour and conservation.

## METHODS

### Scent Sample Collection and Presentation

We conducted four scent presentation experiments on black rhinoceros, *D. b. var. minor*, captured by the Ezemvelo KwaZulu-Natal Wildlife veterinary and game-capture team from KwaZulu-Natal wildlife reserves, South Africa, and held temporarily in captivity at iMfolozi Game Reserve prior to their transport and release into other reserves during 2004–2006 (e.g. Linklater et al. 2006). Rhinoceros subjects were held individually in enclosures approximately 80 m<sup>2</sup> configured in two equal portions, one with an elevated roof as shade, and connected by a short corridor. Animals were fed lucerne hay, pelleted domestic ungulate food and freshly cut browse each morning and evening and water was available ad libitum from troughs (see also Linklater et al. 2010).

We collected dung and urine-soaked sand samples from donor rhinoceros whose sex and age were known because they were part of this or other capture–translocation–release efforts (Linklater et al. 2006; Linklater & Swaisgood 2008). Dung was much easier to locate and collect than urine and so most research focused on dung chemosignals. Control samples comprised river sand collected from the same location as the enclosures' substrate, matching any odour cues deriving from the sand in urine-soaked sand or clinging to dung samples. All samples, each of approximately 300 ml, were sealed in plastic bags and frozen the same day as collection.

For our experiments, each rhino received a maximum of one scent presentation every 2 days (with the exception of the individual discrimination experiment; see below). We conducted presentations in the early morning or evening (0500–0900, 1600–1900 hours) when rhino are most active and avoiding periods when routine husbandry activities (e.g. feeding and cleaning) might interfere with tests. Scent samples were thawed and allowed to reach ambient temperature before presentation. To begin a presentation, we dropped samples into the enclosure from a walkway above it, from which behavioural observations were also made. We recorded all occurrences of sniffing events (bouts separated by 5 s) and the duration of sniffing, where sniffing was operationally

defined as head down, oriented towards the sample and less than one head length from the sample and often confirmed by the movement of nostrils and sound of air passage. This behavioural sampling yielded two dependent variables for analysis: total time spent sniffing and frequency of sniffing events.

### *Experimental Design and Procedures*

We conducted four experiments to evaluate black rhinoceros's ability to discriminate between odour cues in potential chemosignals. In experiment 1 we conducted a habituation–dishabituation experiment (Halpin 1986; Swaisgood et al. 1999) to determine whether subjects could discriminate between odours from different individuals. Dung samples from 10 individuals housed separately at iMfolozi were balanced for donor age and sex and presented to eight subjects (four adult males, four adult females) in distant pens. Only one behavioural sample was collected from an individual rhinoceros during a 24 h period. The habituation phase consisted of presenting a sample from the same dung, and therefore individual, on 3 consecutive days, for which we predicted a gradually diminishing olfactory response (i.e. habituation). On the fourth day we presented the dung from a novel individual, matched to the sex–age class of the habituation-phase donor, predicting an increased olfactory response (dishabituation). We conducted half of the control presentations of river sand before (day 0) this experiment and half after (day 5), balanced between the subject sexes.

In experiment 2 we sought to determine whether rhinoceros overtly discriminated age and sex odour cues present in dung. Samples collected from adult (>6 years of age) and subadult (2–4 years of age) and male and female donors from four southern African reserves (Linklater & Swaisgood 2008) were presented to the same subjects in a randomized order. Scent exemplars were collected from 20 individuals and presented to three to eight adult male subjects and three to nine adult female subjects. Variability in sample size for different scent tests (see Results) was dictated by the availability of scent stimuli from donors of different sex–age classes at the time of the experiment and availability of adult subjects.

Most of our experiments relied on readily available dung, but in experiment 3 we sought to determine whether urine also functions as a chemosignal in black rhinoceros. We tested subject response to dung versus urine to determine whether urine elicited similar olfactory investigation patterns as dung. Dung and urine samples from the same six adult males and females captured and housed at iMfolozi were randomly assigned to adult subjects ( $N = 2–9$ ; see Results), while ensuring that individual subjects did not receive dung and urine from the same donor.

In experiment 4 we evaluated whether black rhinoceros showed overt discrimination of dung aged for various lengths of time and also sought to determine its biological longevity, that is, how long dung continues to elicit a detectable response. For this experiment, dung samples collected from individuals living in the same Namibian reserve (reserve P7 in Linklater & Swaisgood 2008) were aged under conditions similar to those encountered in iMfolozi Game Reserve where the study was conducted. Our intent was to expose dung samples to variation in insect activity, temperature and humidity to allow for the decay of chemical signals present in dung. For this study, we collected five samples from three defecation events (piles) from different rhinoceros, placed them in polystyrene cups under shade outside at ambient temperature at the study site, and aged them in a wooden box for 1, 4, 16 and 32 days. The study was conducted during the dry season and thus samples were not subjected to any ageing effects of rainfall. By minimizing the effects of some variables that may affect chemosignal decay (direct sunlight, rainfall), we expected these samples to retain chemical

information of interest to investigating rhinoceros for longer than if they were exposed to harsher climatic conditions. A sixth sample was retained frozen to represent a fresh dung sample (0 days ageing). In this way samples from three donor rhinoceros were collected and presented to four adult female and one adult male subjects. Sample presentation order was random with regard to experimental treatment (dung age) and 1–26 days (average  $\pm 1SD = 5 \pm 7$  days) separated presentations to the same individual rhinoceros to minimize habituation effects.

### *Statistical Analyses*

We analysed all four studies with a one-way or repeated measures MANOVA of the two dependent variables using the multivariate general linear models procedure in SPSS (SPSS Inc., Chicago, U.S.A.). For the habituation–dishabituation experiment, we used repeated measures MANOVA where the presentation sequence on the same rhino described the repeated measure and post hoc paired Student's *t* tests to discriminate between dyads in the sequence. Tests for the age–sex discriminatory ability of rhinoceros and their differential responses to dung and urine incorporated subject sex and donor age and sex as fixed effects, and tested for their interaction. Levene's and Box's tests for equality of variance and the covariance matrix for each MANOVA revealed inequalities that were addressed by natural logarithmic transformation of the dependent variables. We considered test statistics as significant with *P* values <0.05. All analyses were carried out with SPSS version 16.0.

### *Ethical Note*

All procedures were approved by The Institutional Animal Care and Use Committee of the San Diego Zoo Institute for Conservation Research (U.S.A.) Permit Number 169, and Ezemvelo KwaZulu-Natal Wildlife, South Africa, field research permits ZC/101/01 and ZC/097/05.

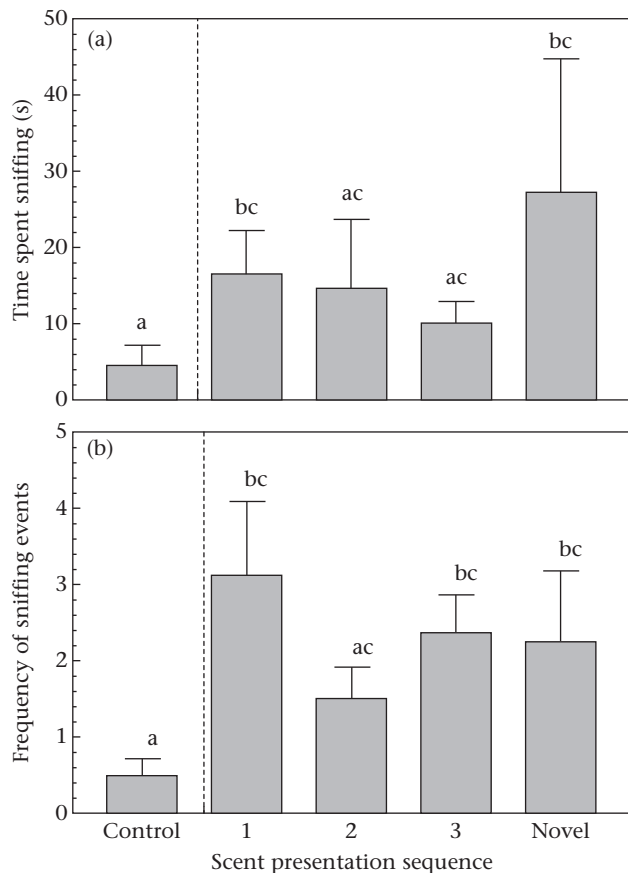
## RESULTS

### *Experiment 1: Individual Odour Discrimination of Dung*

Black rhinoceros demonstrated the expected declining response to presentations of the scent from the same donor on consecutive days (habituation) and increased investigation when a novel scent from a different individual was presented on the fourth day (dishabituation). The omnibus *F* test was not significant (MANOVA:  $F_{6,42} = 1.04$ ,  $P = 0.41$ ; Fig. 1), as might be expected if the habituation effect were strong each consecutive day of presentation. Post hoc paired tests revealed significant differences between the control and the first dung presentation, suggesting clear discrimination of this conspecific odour (Fig. 1). As expected, rhinos did not overtly discriminate between controls and the second and third presentations of scent from the same donor (habituation). During the dishabituation trial, however, there was again a significant discrimination between the control and the novel dung presentation, providing evidence that the subjects could discriminate between the faecal odour of different rhinos.

### *Experiment 2: Sex and Age Discrimination of Dung*

There were significant effects of donor sex and age on the olfactory investigation of dung (MANOVA: donor sex:  $F_{4,78} = 4.66$ ,  $P = 0.002$ ; donor age:  $F_{4,108} = 3.01$ ,  $P = 0.021$ ; Fig. 2), but no interaction between them and subject sex (MANOVA: donor–subject sex:  $F_{4,78} = 0.57$ ,  $P = 0.816$ ; donor age–subject sex:  $F_{4,110} = 0.637$ ,



**Figure 1.** Olfactory investigation behaviour of dung. (a) Time spent sniffing and (b) frequency of sniffing events by eight individuals. Each individual received dung from a different donor. The donor's dung was presented to the same subject on each of 3 consecutive days (1–3) followed by the presentation of a sample of dung from another different, but same-sex, individual (novel). Investigation behaviours in response to control presentations of enclosure sand substrate (control) made before or after the dung presentation sequence in equal number are also shown. Significant differences (from pairwise, post hoc significance tests) are indicated by different letters (a–c) above each bar. Bars represent averages + 1 SE.

$P = 0.637$ ). Statistical interactions indicate that male and female subjects discriminated similarly between male and female scents (MANOVA:  $F_{2,38} = 1.93$ ,  $P = 0.16$ ). In contrast, male and female subjects responded differently when presented with adult versus subadult dung (MANOVA interaction:  $F_{2,54} = 3.36$ ,  $P = 0.035$ ; Fig. 2).

#### Experiment 3: Dung versus Urine

We found little evidence that black rhinoceros show investigatory preferences between dung and urine, indicating that both conspecific odours are likely to play a role in communication (MANOVA: dung versus urine:  $F_{2,42} = 1.41$ ,  $P = 0.26$ ; Fig. 3). However, the interaction between donor sex and scent type (dung or urine) had a nearly significant effect on investigation behaviour (MANOVA: donor\*treatment:  $F_{2,42} = 3.14$ ,  $P = 0.054$ ). Dung and urine appeared to be investigated differently depending on the sex of the donor. Male dung received greater interest than female dung but the trend was reversed for urine. Female urine received greater investigation than urine from males.

#### Experiment 4: Discrimination of Dung Scent Age

Black rhino investigation behaviour did not decline with dung aged up to at least 32 days (repeated measures MANOVA:

$F_{2,42} = 1.23$ ,  $P = 0.34$ ; Fig. 4), although dung by this time appeared to consist largely of dry sticks, that is, remnants of browsed twigs. Examination of Fig. 4 suggests that rhinoceros were most responsive to fresh dung and that interest declined thereafter, before (surprisingly) rebounding for dung aged 32 days; however, this pattern is not statistically significant. Low levels of replication prevent us from examining subject sex differences. The single male sniffed dung aged 32 days significantly more often and for longer than the four females (time spent sniffing).

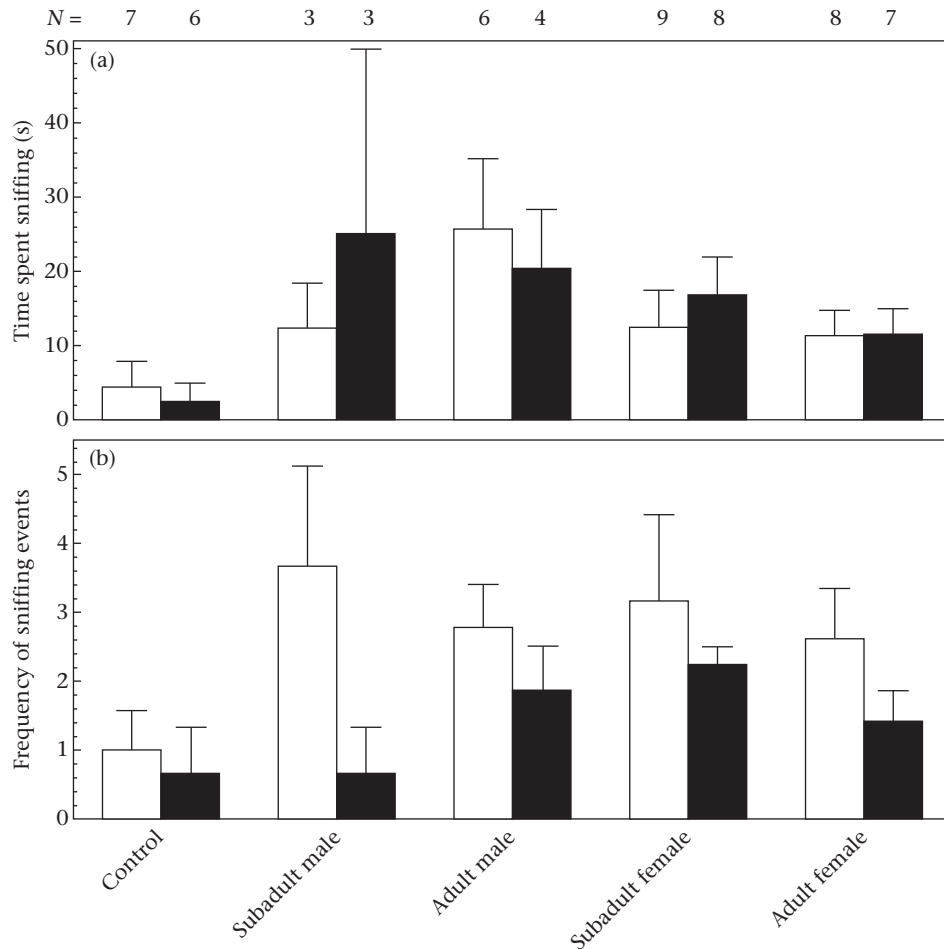
#### DISCUSSION

This series of olfactory discrimination tests in wild-caught temporarily captive black rhinoceros indicate that dung is an important chemosignal in the species. Rhinos readily approached and investigated conspecific dung, displaying differential overt behaviour in response to dung from different classes of individuals. Black rhinos also investigated urinary chemosignals and tended to treat urine and dung from males and females differently. These tests may be considered conservative in that the rhinos were held in less than ideal situations for the expression of these olfactory abilities, that is, while being held in small enclosures where exposure to human and conspecific scent was much greater than would have been encountered in the wild and in circumstances known to activate stress and distress responses (Linklater et al. 2010). None the less, this study suggests that black rhinoceros may use olfactory signals in dung to recognize individual identity, sex and age of the signaller. This general conclusion is consistent with our expectations from the social and spatial organization of black rhinoceros populations. Groups of females are uncommon. Individuals have different home ranges that overlap intersexually. Females' ranges overlap, but the ranges of males overlap only at their boundaries such that some have described males as territorial (Goddard 1967; Mukinya 1973; Kiwia 1989; Tatman et al. 2000; Lent & Fike 2003; Linklater & Hutcherson 2010). Black rhinoceros, therefore, are a solitary-living species that is likely to depend on remote communication.

Our habituation–dishabituation experiment indicates that black rhinos can discriminate individual differences in dung odours. This finding suggests that rhinos produce dung with individual chemical signatures, but more research is needed to determine whether receivers recognize individuals in future encounters. The diminished investigatory response to repeated presentation of dung from the same individual may indicate that social familiarity is important, similar to the outcomes of Goddard's (1967) small field trial. Many species respond more aggressively to unfamiliar same-sex rivals than to familiar or neighbouring rivals, possibly because unfamiliar individuals represent a greater threat to the status quo (Archer 1988). Black rhinos appear more prone to aggressive interaction with unfamiliar than familiar rhinos (Linklater & Swaisgood 2008). Discriminating individual scents is also a precondition for 'scent matching,' in which individuals assess the competitive ability of rivals by matching the scent saturating a territory with the scent of an encountered individual (Gosling & Roberts 2001). Recognizing individuals from olfactory signals, or at least discriminating familiar versus unfamiliar rivals, may be an important communicatory process giving rise to the observed social organization of black rhinoceros, but how the process generates patterns in spatial and temporal organization remains to be determined. Discrimination between opposite-sex individuals using odour cues may also be important for recognizing and selecting potential mates (e.g. Tang-Martinez et al. 1993; Fisher et al. 2003b).

Black rhinos' ability to discriminate the age class and sex of the signaller from odour cues in dung may also play an important role in governing social behaviour. For example, a male may need to





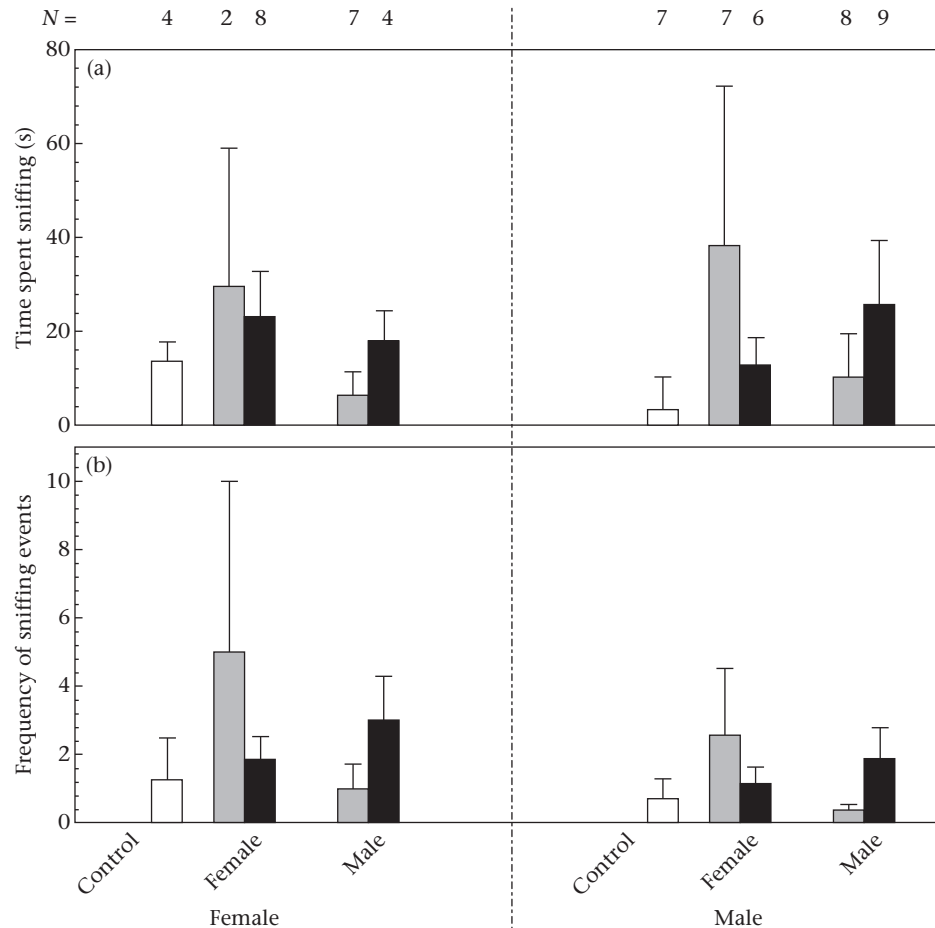
**Figure 2.** Olfactory investigation behaviour of dung by adult (i.e.  $\geq 6$  year old) female (white bar) and male (black bar) black rhinoceros. (a) Time spent sniffing and (b) frequency of sniffing events. Donors were subadult (i.e. 2–4 years old) and adult females and males. Bars represent averages  $\pm 1$  SE.

assess signaller age from dung to determine the degree of threat posed by a potential rival or to evaluate whether a female is old enough to be a potential mate, and avoid pursuit of immature females. Chemical signals may be particularly important to assess rivals because they enable the receiver to gather information about the signaller without risking a direct encounter. Both males and females showed similar response patterns to dung from males versus females, with both sexes displaying more investigatory behaviour in response to male dung. This finding may indicate the importance of male–male competition in black rhinoceros, while females' preferential investigation of male dung may indicate assessment of mate quality. By contrast, males and females responded differently to adult versus subadult dung. Examination of Fig. 2 suggests that subadult females' dung was investigated more than adult female dung by both sexes. The response to subadult male dung, however, was more complex. Adult females sniffed it more often but only briefly each time such that the total time sniffing was comparatively low. Adult males treated subadult male dung in the opposite way. They visited it fewer times, but could spend an exceptionally long time sniffing, a finding also consistent with the interpretation that both sexes use chemical communication mostly as a tool for identifying potential mates but males also use chemical communication to govern competitive interactions. Nevertheless, we caution that this apparent interaction between donor age and sex and subject sex was not supported statistically, perhaps because of the limited number of subadult male samples.

Black rhinos investigated both dung and urine more than control stimuli, indicating that both odour sources play a role in chemosignalling. Importantly, male dung was investigated more than male urine by both sexes, but rhinos investigated female urine more than female dung (Fig. 3), contributing to the conclusion that urine is the more important chemical signal produced by females. These interpretations from olfactory discrimination tests need to be confirmed with observations of wild rhino competitive and reproductive behaviour to determine the link between signal, response, and intra- and intersexual relationships.

Rhinos failed to discriminate between dung aged for various lengths of time up to 32 days. Despite visual indicators of faecal decay, rhinos remained as responsive to month-old dung as fresh dung. While olfactory discrimination tests such as ours only allow us to examine investigatory differences, it is possible that rhinos still extract different information from dung of various ages and, in a natural setting, may follow different courses of action subsequently. That dung should remain biologically active for 1 month is not surprising; other species will continue to investigate scent aged for more than 3 months, albeit at a reduced rate (Johnston & Schmidt 1979; Swaisgood et al. 2004).

These olfactory discrimination patterns indicating the relative importance of dung and urine in chemical communication by male and female black rhinoceros are also consistent with observations of urine- and dung-marking behaviour (Schenkel & Schenkel-Hulliger 1969; Owen-Smith 1988). Adult males, but not females or subadult males, scrape the ground and kick the dung backwards

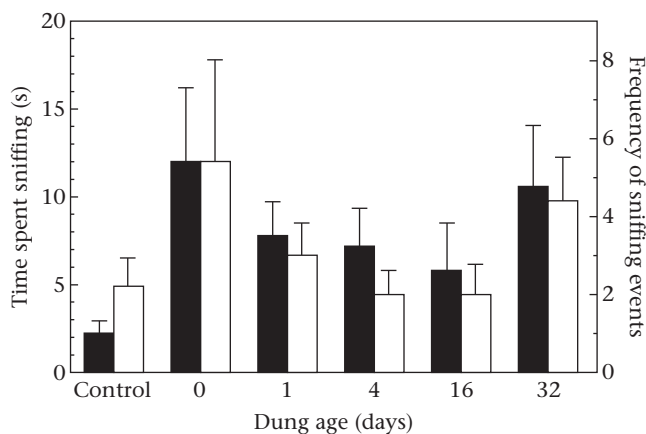


**Figure 3.** Olfactory investigation behaviour of urine (grey bar), dung (black bar) and the sand control (white bar) by adult (i.e.  $\geq 6$  year old) male and female black rhinoceros. (a) Time spent sniffing and (b) frequency of sniffing events. Bars represent averages  $\pm 1$  SE.

and forwards spreading it several metres, making the dung more visible and probably increasing the size of the odour field, aiding in signal detection (*sensu* Alberts 1992). Males' dung is also frequently deposited at the base of a bush and branches may be broken. Similarly, adult males spray urine frequently and they often appear to target bushes or other elevated objects, and females will urinate

small volumes frequently when in oestrus. Thus, both defecation and urination appear ritualized for signal function.

Although this study is somewhat limited in scope, we have shown that olfactory communication, particularly with dung, is important in black rhinoceros, and have illuminated some of the behavioural processes often overlooked in favour of describing the product (i.e. social and spatial pattern). Understanding behavioural processes such as these are critical for the nascent field of conservation behaviour because mechanisms can be manipulated, whereas functions cannot (Linklater 2004). Others have advanced the use of communication research for conservation management (Fisher et al. 2003a; Reed 2004; Roberts & Gosling 2004; Swaisgood 2007; Swaisgood & Schulte 2010; Campbell-Palmer & Rosell 2011) and it is a promising tool for managing the behaviour of black rhinoceros following translocation (Linklater et al. 2006). Translocation is essential for re-establishing black rhinoceros to protected areas, genetic rescue and metapopulation management. Dung is easily acquired and distributed, appears important for regulating social behaviour, and remains biologically active for at least a month. These observations raise the possibility of using dung broadcasting in the postrelease environment to modulate aggression after restocking populations or encourage their settlement safely into new areas, in the same way that song playback has been used to establish settlement of birds into unoccupied areas (reviewed in Ahlering et al. 2010). Black rhino breeding in captivity is still considered suboptimal (Foote & Wiese 2006) and communication problems could be a contributory factor to failed courtship.



**Figure 4.** Olfactory investigation behaviours measured as time spent sniffing (black bar) and frequency of sniffing events (white bar) by four adult females and one adult male in response to the random order presentation of fresh dung and dung from the same rhino aged 1, 4, 16 and 32 days. Bars represent averages  $\pm 1$  SE.

Black rhino chemosignals could be manipulated to promote successful mating, as has been shown in other species with similar life history characteristics (Fisher et al. 2003a, b; Swaisgood et al. 2004). Future research should explore the utility of using dung chemosignals to modify black rhino behaviour to support conservation management of the species. The relationships between signal, response and social outcomes remain to be demonstrated for black rhino. Manipulations of olfactory signalling for conservation might not only yield new tools but also be grand landscape-scale experiments to improve our understanding of olfactory communication in this otherwise difficult-to-study species.

## Acknowledgments

Our thanks to staff of Ezemvelo KwaZulu-Natal Wildlife, particularly Quinton Rochat, Sue van Rensburg, Craig Reid, Jeff Cook and Ilse Schmit, for their assistance. This research was funded by San Diego Zoo Global and the U.S. Fish and Wildlife Service Rhinoceros and Tiger Conservation Fund (grant agreement numbers 98210-2-G363, 98210-4-G920, and 98210-6-G102). Our thanks to Graham Kerley (ACE-NMMU), Jacques Flammand (WWF-Black Rhinoceros Range Expansion Project), and Alan Dixson (San Diego Zoo Global) for their support, and Angela White, Kirsten Bond, Dale Airton, Chris Kelly for their contributions to field work.

## References

- Ahlering, M., Arlt, D., Betts, M., Fletcher, R., Nocera, J. & Ward, M. 2010. Research needs and recommendations for the use of conspecific-attraction methods in the conservation of migratory songbirds. *Condor*, **112**, 252–264.
- Alberts, A. C. 1992. Constraints on the design of chemical communication systems in terrestrial vertebrates. *American Naturalist*, **139**, S62–S89.
- Archer, J. 1988. *The Behavioural Biology of Aggression*. Cambridge: Cambridge University Press.
- Barnard, C. & Burk, T. 1979. Dominance hierarchies and the evolution of 'individual recognition'. *Journal of Theoretical Biology*, **81**, 65–73.
- Borner, M. 1979. A field study of the Sumatran rhinoceros *Dicerorhinus sumatrensis* Fischer 1814. Ph.D. thesis, University of Basel.
- Bronson, F. 1989. *Mammalian Reproductive Biology*. Chicago: University of Chicago Press.
- Brown, R. E. 1979. Mammalian social odors: a critical review. *Advances in the Study of Behavior*, **10**, 103–162.
- Campbell-Palmer, R. & Rosell, F. 2011. The importance of chemical communication studies to mammalian conservation biology: a review. *Biological Conservation*, **144**, 1919–1930.
- Carlstead, K., Mellen, J. & Kleiman, D. G. 1999. Black rhinoceros (*Diceros bicornis*) in US zoos: I. Individual behavior profiles and their relationship to breeding success. *Zoo Biology*, **18**, 17–34.
- Connor, J. 1972. Olfactory control of aggressive and sexual behavior in the mouse (*Mus musculus*). *Psychonomic Science*, **27**, 1–3.
- Dixson, A. & Mackintosh, J. 1971. Effects of female urine upon the social behaviour of adult male mice. *Animal Behaviour*, **19**, 138–140.
- Eisenberg, J. & Kleinman, D. 1972. Olfactory communication in mammals. *Annual Review of Ecology and Systematics*, **3**, 1–32.
- Fisher, H., Swaisgood, R. & Fitch-Snyder, H. 2003a. Odor familiarity and female preferences for males in a threatened primate, the pygmy loris *Nycticebus pygmaeus*: applications for genetic management of small populations. *Naturwissenschaften*, **90**, 509–512.
- Fisher, H., Swaisgood, R. & Fitch-Snyder, H. 2003b. Countermarking by male pygmy lorises (*Nycticebus pygmaeus*): do females use odor cues to select mates with high competitive ability? *Behavioral Ecology and Sociobiology*, **53**, 123–130.
- Foose, T. & Wiese, R. 2006. Population management of rhinoceros in captivity. *International Zoo Yearbook*, **40**, 174–196.
- Goddard, J. 1967. Home range, behaviour, and recruitment rates of two black rhinoceros populations. *East African Wildlife Journal*, **5**, 133–150.
- Gosling, L. M. & Roberts, S. C. 2001. Scent-marking by male mammals: cheat-proof signals to competitors and mates. *Advances in the Study of Behavior*, **30**, 169–217.
- Halpin, Z. 1986. Individual odors among mammals: origins and functions. *Advances in the Study of Behavior*, **16**, 39–70.
- Johnston, R. & Schmidt, T. 1979. Responses of hamsters to scent marks of different ages. *Behavioral and Neural Biology*, **26**, 64–75.
- Joubert, E. & Eloff, F. C. 1971. Notes on the ecology and behaviour of the black rhinoceros *Diceros bicornis* Linn. 1758 in South West Africa. *Madoqua*, **1**, 5–53.
- Kiwi, H. 1989. Ranging patterns of the black rhinoceros *Diceros bicornis* L. in Ngorongoro Crater Tanzania. *African Journal of Ecology*, **27**, 305–312.
- Laurie, A. 1982. Behavioural ecology of the greater one-horned rhinoceros (*Rhinoceros unicornis*). *Journal of Zoology*, **196**, 307–341.
- Lent, P. C. & Fike, B. 2003. Home ranges, movements and spatial relationships in an expanding population of black rhinoceros in the Great Fish River Reserve, South Africa. *South African Journal of Wildlife Research*, **33**, 109–118.
- Linklater, W. 2003. Science and management in a conservation crisis: a case study with rhinoceros. *Conservation Biology*, **17**, 968–976.
- Linklater, W. 2004. Wanted for conservation research: behavioral ecologists with a broader perspective. *BioScience*, **54**, 352–360.
- Linklater, W. & Hutchesson, I. 2010. Black rhinoceros are slow to colonize a harvested neighbour's range. *South African Journal of Wildlife Research*, **40**, 58–63.
- Linklater, W. & Swaisgood, R. 2008. Reserve size, release density and translocation success: black rhinoceros movements, association, injury and death after release. *Journal of Wildlife Management*, **72**, 1059–1068.
- Linklater, W., Flammand, J., Rochet, Q., Zekela, N., MacDonald, E., Swaisgood, R., Airton, D., Kelly, C., Bond, K., Schmidt, I., et al. 2006. Preliminary analyses of the free-release and scent-broadcasting strategies for black rhinoceros reintroduction. *Ecological Journal, Conservation Corporation Africa*, **7**, 26–34.
- Linklater, W., MacDonald, E., Flammand, J. & Czekala, N. 2010. Declining and low fecal corticoids are associated with distress, not acclimation to stress, during the translocation of African rhinoceros. *Animal Conservation*, **13**, 104–111.
- Linklater, W., Adcock, K., du Preez, P., Swaisgood, R., Law, P., Knight, M., Gedir, J. & Kerley, G. 2011. Guidelines for large herbivore translocation simplified: black rhinoceros case study. *Journal of Applied Ecology*, **48**, 493–502.
- Linklater, W., Gedir, J., Law, P., Swaisgood, R., Adcock, K., du Preez, P., Knight, M. & Kerley, G. 2012. Translocations as experiments in the ecological resilience of an asexual mega-herbivore. *PLoS One*, **7**, e30664.
- Mossman, C. & Drickamer, L. 1996. Odor preferences of female house mice (*Mus domesticus*) in seminatural enclosures. *Journal of Comparative Psychology*, **110**, 131–138.
- Mukinya, J. T. 1973. Density, distribution, population structure and social organization of the black rhinoceros in Masai Mara Game reserve. *East African Wildlife Journal*, **11**, 385–400.
- Muller-Schwarze, D. 2006. *The Chemical Ecology of Vertebrates*. Cambridge: Cambridge University Press.
- Nie, Y., Swaisgood, R., Zhang, Z. & Wei, F. 2012. Giant panda scent marking strategies in the wild: role of season, sex and marking surface. *Animal Behaviour*, **84**, 39–44.
- Owen-Smith, R. N. 1973. The behavioral ecology of the white rhinoceros. Ph.D. thesis, University of Wisconsin.
- Owen-Smith, R. N. 1988. *Mega-herbivores: The Influence of Large Body Size on Ecology*. Cambridge: Cambridge University Press.
- Reed, J. 2004. Recognition behavior based problems in species conservation. *Annales Zoologici Fennici*, **41**, 859–877.
- Regnier, F. & Goodwin, M. 1977. On the chemical and environmental modulation of pheromone release from vertebrate scent marks. In: *Chemical Signals in Vertebrates* (Ed. by D. Müller-Schwarze & M. Mozell), pp. 115–133. New York: Plenum.
- Roberts, S. C. 1998. Behavioural responses to scent marks of increasing age in klipspringer *Oreotragus oreotragus*. *Ethology*, **104**, 585–592.
- Roberts, S. & Gosling, L. 2004. Manipulation of olfactory signaling and mate choice for conservation breeding: a case study of harvest mice. *Conservation Biology*, **18**, 548–556.
- Schenkel, R. & Schenkel-Hulliger, L. 1969. *Ecology and Behaviour of the Black Rhinoceros (Diceros bicornis L.): a Field Study*. Hamburg: Verlag Paul Harvey.
- Sherman, P., Reeve, H. & Pfennig, D. 1997. Recognition systems. In: *Behavioural Ecology: an Evolutionary Approach* (Ed. by J. Krebs & N. Davies), pp. 69–96. Oxford: Blackwell Science.
- van Strien, N. 1986. *The Sumatran Rhinoceros, Dicerorhinus sumatrensis, in the Gunung Leuser National Park, Sumatra, Indonesia: Its Distribution, Ecology and Conservation*. Hamburg: Paul Parey.
- Swaisgood, R. 2007. Current status and future directions of applied behavioral research for animal welfare and conservation. *Applied Animal Behaviour Science*, **102**, 139–162.
- Swaisgood, R. & Schulte, B. 2010. Applying knowledge of mammalian social organization, mating systems and communication to management. In: *Wild Mammals in Captivity* (Ed. by D. Kleiman, K. Thompson & C. Baer), pp. 329–343. Chicago: University of Chicago Press.
- Swaisgood, R. R., Lindburg, D. G. & Zhou, X. P. 1999. Giant pandas discriminate individual differences in conspecific scent. *Animal Behaviour*, **57**, 1045–1053.
- Swaisgood, R., Lindburg, D., Zhou, X. & Owen, M. 2000. The effects of sex, reproductive condition and context on discrimination of conspecific odours by giant pandas. *Animal Behaviour*, **60**, 227–237.
- Swaisgood, R., Lindburg, D., White, A., Zhang, H. & Zhou, X. 2004. Chemical communication in giant pandas: experimentation and application. In: *Giant Pandas: Biology and Conservation* (Ed. by D. Lindburg & K. Baragona), pp. 106–120. Berkeley, California: University of California Press.
- Tang-Martinez, Z., Mueller, L. & Taylor, G. 1993. Individual odors and mating success in the golden hamster, *Mesocricetus auratus*. *Animal Behaviour*, **45**, 1141–1151.
- Tatman, S. C., Stevens-Wood, B. & Smith, V. B. T. 2000. Ranging behaviour and habitat usage in black rhinoceros, *Diceros bicornis*, in a Kenyan sanctuary. *African Journal of Ecology*, **38**, 163–172.
- Tibbetts, E. & Dale, J. 2007. Individual recognition: it is good to be different. *Trends in Ecology & Evolution*, **22**, 529–537.
- Wyatt, T. 2003. *Pheromones and Animal Behaviour: Communication by Smell and Taste*. Cambridge: Cambridge University Press.